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# Use of 25-hydroxyvitamin D<sub>3</sub> and vitamin E to improve tenderness of beef from the longissimus dorsi of heifers<sup>1,2</sup>

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**ABSTRACT:** The objective of this trial was to determine whether a single bolus of 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>), vitamin E, or a combination of the 2 would improve the tenderness of steaks from the LM of beef heifers. Forty-eight Angus crossbred heifers were allotted randomly to 8 pens. Six heifers were in each pen, and there were 2 pens per treatment. The 4 treatments included control (no 25-OH D<sub>3</sub> or vitamin E); 25-OH D<sub>3</sub> (500 mg of 25-OH D<sub>3</sub> administered as a one-time oral bolus 7 d before slaughter); vitamin E (1,000 IU of vitamin E administered daily as a top-dress for 104 d before slaughter); or combination (500 mg of 25-OH D<sub>3</sub> administered as a one-time oral bolus 7 d before slaughter and 1,000 IU of vitamin E administered daily as a top-dress for 104 d before slaughter). Blood samples were obtained on the day that heifers were allotted to treatments, on the day 25-OH D<sub>3</sub> was administered, and on the day before slaughter. Plasma calcium concentration was increased when 25-OH D<sub>3</sub> was administered with

or without vitamin E ( $P < 0.007$ ). In LM, calcium concentration tended to increase ( $P = 0.10$ ) when 25-OH D<sub>3</sub> was administered alone but not when 25-OH D<sub>3</sub> was administered with vitamin E. Concentrations of 25-OH D<sub>3</sub> and 1,25-dihydroxyvitamin D<sub>3</sub> in plasma were increased when 25-OH D<sub>3</sub> was administered with or without vitamin E ( $P < 0.001$ ). Steaks from heifers treated with 25-OH D<sub>3</sub> or vitamin E, but not both, tended to have lower Warner-Bratzler shear force than steaks in the control group at 14 d postmortem ( $P = 0.08$ ). Postmortem protein degradation as measured by Western blot of the 30-kDa degradation product of troponin-T was increased with all treatments after 3 d postmortem ( $P \leq 0.07$ ), but not at 7 or 14 d postmortem. Unexpectedly, the use of 500 mg of 25-OH D<sub>3</sub> fed as an oral bolus 7 d before slaughter or 1,000 IU of vitamin E administered daily for 104 d before slaughter alone, but not in combination, effectively decreased Warner-Bratzler shear force.

**Key words:** beef, 25-hydroxyvitamin D<sub>3</sub>, tenderness, vitamin E

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## INTRODUCTION

Tenderness is one of the most important quality characteristics affecting the beef industry in the United

States. In fact, inconsistent tenderness costs the beef industry millions of dollars annually (Smith et al., 1995). Consumers do distinguish between tenderness categories (Boleman et al., 1997) and are willing to pay a premium for meat that is guaranteed to be tender (Shackelford et al., 2001). Recently, research has focused on a practical, on-the-farm method of producing consistently tender beef that would result in increased profits.

Researchers have demonstrated that feeding a supra-natural dosage (0.5 to 7.5 million IU) of vitamin D<sub>3</sub> to beef cattle for 7 to 10 d before slaughter results in more tender beef (Swanek et al., 1999; Montgomery et al., 2000, 2002; Karges et al., 2001). Feeding these dosages of vitamin D<sub>3</sub> results in elevated calcium concentrations in plasma and beef and improved tenderness, presumably by enhanced action of calcium-dependent prote-

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ases, m- and  $\mu$ -calpain, on myofibrillar protein degradation postmortem (Koohmaraie, 1992).

The 25-hydroxyvitamin D<sub>3</sub> (**25-OH D<sub>3</sub>**) seems to elicit responses similar to those of vitamin D<sub>3</sub> without high residues of vitamin D<sub>3</sub> or its metabolites in beef. Feeding a supranatural amount of 25-OH D<sub>3</sub> has caused favorable changes in meat characteristics, but improved tenderness has not resulted (Foote et al., 2004; Wertz et al., 2004). The dosage of 25-OH D<sub>3</sub> was not sufficient to elevate muscle calcium concentration in these studies.

Recent data suggest that the antioxidant characteristics of vitamin E fed at 1,000 IU/d for 125 d before slaughter may protect the calpains from oxidation (Rowe et al., 2004). By combining these 2 research foci of vitamins D and E, we hypothesized that feeding 25-OH D<sub>3</sub> and vitamin E in combination would result in elevated plasma and muscle calcium concentration and protection for the calpains so that maximal tenderness could be achieved with minimal accumulation of vitamin D<sub>3</sub> metabolites in the muscle.

To test our hypothesis, we designed a study to examine the effect of 25-OH D<sub>3</sub>, vitamin E, and a combination of the 2 on tenderness of beef from the LM of market weight beef heifers.

## MATERIALS AND METHODS

Approval for this project was obtained from the Iowa State University Animal Care and Use Committee, and all regulations were followed.

Forty-eight 13-mo-old Angus crossbred beef heifers, obtained from a breeding project at Iowa State University, were housed at the Iowa State University Beef Nutrition Research Farm in Ames, IA. Heifers were allotted randomly to 1 of 4 dietary treatments (control, no 25-OH D<sub>3</sub> or vitamin E supplementation; 25-OH D<sub>3</sub>, 500 mg of 25-OH D<sub>3</sub> administered once as an oral bolus 7 d before slaughter; vitamin E, 1,000 IU of vitamin E administered daily for 104 d before slaughter; and combination, 500 mg of 25-OH D<sub>3</sub> administered as an oral bolus 7 d before slaughter and 1,000 IU of vitamin E administered daily for 104 d before slaughter). Heifers were housed in 8 pens of 6 heifers, and all heifers in each pen were in the same dietary treatment. Two pens were allotted to each treatment. The vitamin E (Rovimix E-50 Adsorbate) and 25-OH D<sub>3</sub> (Rovimix Hy-D 1.25) were obtained from DSM Nutritional Products Inc., Ames, IA. The vitamin E was mixed with soybean meal to provide 6,000 IU of vitamin E daily per pen. The soybean meal containing vitamin E or soybean meal alone, for the control and 25-OH D<sub>3</sub> groups, was spread evenly over the feed each day for 104 d before slaughter. The base diet on an as-fed basis was composed of 79% cracked corn, 15% corn silage, 2.6% soybean meal, 1% steep liquor, 0.9% cane molasses, 0.6% urea, 0.5% limestone, and 0.3% sodium chloride. Vitamin A was added at 300 IU/kg of DM, trace minerals were added at 0.02% of DM, and monensin (Rumensin, Elanco Animal

Health, Greenfield, IN) was added at 24 mg/kg. Dosages of 25-OH D<sub>3</sub> or cornstarch (control) were weighed and divided into 5 gelatin capsules. Five capsules were used to ensure that if a heifer unknowingly regurgitated one of the capsules, most of the dosage would remain in the rumen. No regurgitated capsules were found after administration of the boluses. The capsules were administered 7 d before slaughter.

Blood samples were obtained by jugular venipuncture on the day that heifers were assigned to treatments, on the day 25-OH D<sub>3</sub> was administered, and on the day before heifers were sent to the abattoir. Blood was collected by using 3.81-cm, 20-ga needles and heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood samples were stored immediately on ice until centrifugation. Plasma was stored at -20°C until analyses. Heifers were transported 64 km to be slaughtered at the Iowa Quality Beef meat packing plant in Tama, IA. Rib sections were obtained after 48 h and brought to the Iowa State University Meat Laboratory, Ames, IA, for further processing. The next day, rib sections were boned and sliced into six 2.54-cm steaks; each steak was vacuum-packaged individually. Steaks were randomly allotted to aging periods of 3, 7, or 14 d postmortem. Two steaks from each heifer immediately were frozen at -20°C. Those steaks were considered aged for 3 d. The remaining 4 steaks from each heifer were refrigerated at 4°C until 7 or 14 d of aging and then frozen at -20°C. Therefore, 2 steaks from each heifer were aged for 3, 7, and 14 d. One steak from each aging period was used for biochemical analyses, and the other was cooked and used for Warner-Bratzler shear force analysis.

### Calcium Concentration in Plasma and in Muscle

Plasma and meat calcium concentrations were determined by atomic absorption. Briefly, 5 mL of 0.1% lanthanum oxide was added to 100  $\mu$ L of plasma from each heifer; the mixture was vortexed and analyzed by using atomic absorption spectroscopy (AOAC, 1990; Perkin-Elmer Corp., Norwalk, CT). Meat samples were prepared for total calcium analysis by weighing 3 g of muscle into an acid-washed beaker and then using a modified HNO<sub>3</sub>-H<sub>2</sub>SO<sub>4</sub> wet combustion method (NMAM, 1994). The resulting solution was standardized to 25 mL with distilled deionized water and was then analyzed by using atomic absorption spectroscopy (Perkin-Elmer Corp.).

### 25-OH D<sub>3</sub> and 1,25-Dihydroxyvitamin D<sub>3</sub> in Plasma and in Muscle

The 25-OH D<sub>3</sub> in plasma was extracted with acetonitrile and quantified by RIA using <sup>125</sup>I as the tracer (Hollis et al., 1993); the intraassay CV was 6.7%, and the interassay CV was 12.9%. Similarly, 1,25-dihydroxyvitamin D<sub>3</sub> [**1,25-(OH)<sub>2</sub> D<sub>3</sub>**] was extracted by using acetonitrile, but that extract was treated with sodium

**Table 1.** Effect of dietary supplementation with 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>), vitamin E, or both on feedlot performance of beef heifers<sup>1</sup>

Item	Treatment				SEM
	Control	25-OH D <sub>3</sub>	Vitamin E	25-OH D <sub>3</sub> + vitamin E	
Initial wt, kg	359	357	392	389	29
Final wt, kg	536	516	546	520	10
ADG, kg/d	1.38	1.21	1.48	1.26	0.11
DMI, kg/d	8.45	8.54	9.03	8.43	0.35
DMI, <sup>2</sup> kg/d	7.33	6.35	7.05	5.91	0.32
G:F	0.16	0.14	0.16	0.15	0.03

<sup>1</sup>Means represent the average for each treatment (2 pens per treatment, and 6 heifers per pen).

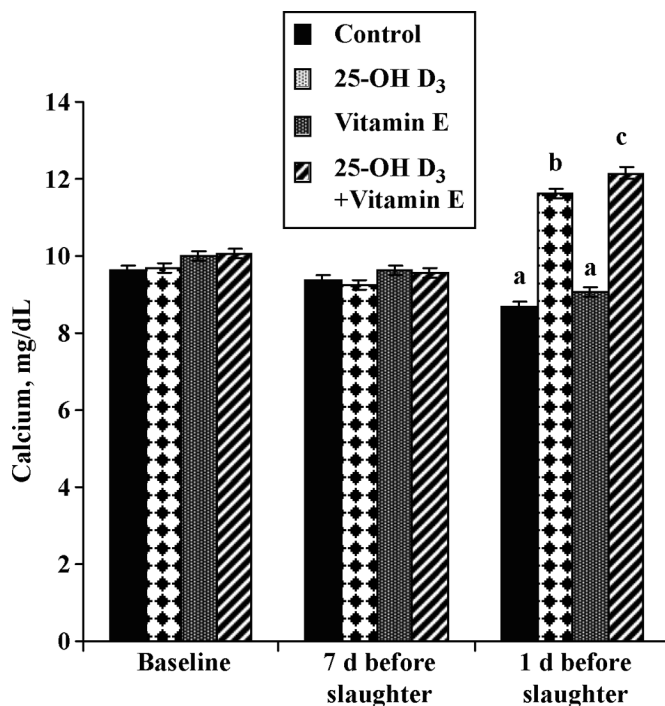
<sup>2</sup>DMI during the last week before slaughter.

periodate and purified by using solid phase extraction. The 1,25-(OH)<sub>2</sub> D<sub>3</sub> was quantified from the purified sample by RIA using <sup>125</sup>I as the tracer (Hollis et al., 1996); the intraassay CV was 7.1%, and the interassay CV was 14.2%. The detection limit of the assay was 2.4 ng/L.

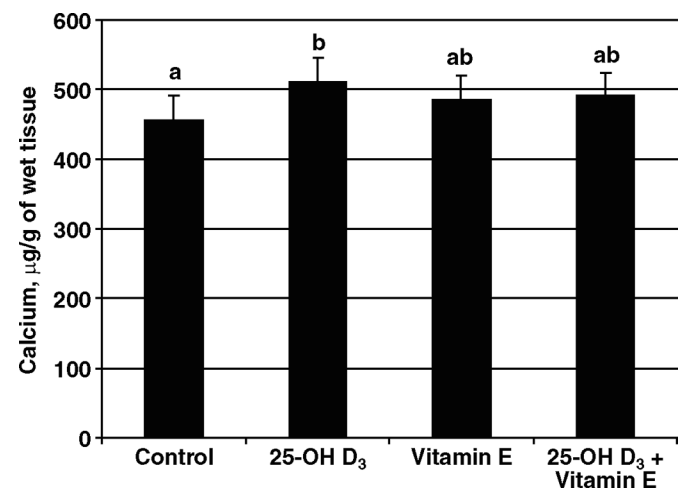
The 25-OH D<sub>3</sub> and 1,25-(OH)<sub>2</sub>D<sub>3</sub> concentrations in meat were determined by using a series of extractions, HPLC, and RIA (Horst et al., 1981).

### Vitamin E in Muscle

To determine whether vitamin E administered to heifers in the vitamin E and combination treatment groups accumulated in the muscle of those animals, vitamin E concentration in meat was determined by analysis with HPLC. Three grams of meat was homogenized in 12 mL of PBS. This homogenate (1 mL) was used for vitamin E analysis.  $\alpha$ -Tocopherol (Sigma, St. Louis, MO) was used as the external standard. Dilutions of 0 to 1,200 ng of vitamin E were used to validate the assay. Retinyl acetate (125 ng) was added to each aliquot as the internal standard. Samples were ex-

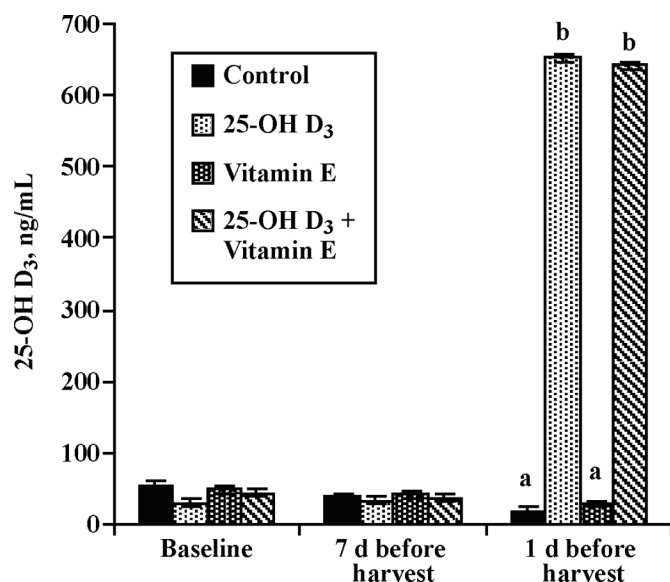


**Figure 1.** Plasma calcium concentration for heifers in control group [no 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>) or vitamin E], 25-OH D<sub>3</sub> group (500 mg of 25-OH D<sub>3</sub> as an oral bolus 7 d before slaughter), vitamin E group (1,000 IU of vitamin E for 104 d before slaughter), or 25-OH D<sub>3</sub> + vitamin E group (25-OH D<sub>3</sub> and vitamin E). No differences ( $P > 0.05$ ) exist at the beginning of the study (baseline) or on the day that 25-OH D<sub>3</sub> was administered (7 d before slaughter). Error bars represent SEM. <sup>a-c</sup>Means from 1 d before slaughter not bearing a common letter differ ( $P \leq 0.05$ ).

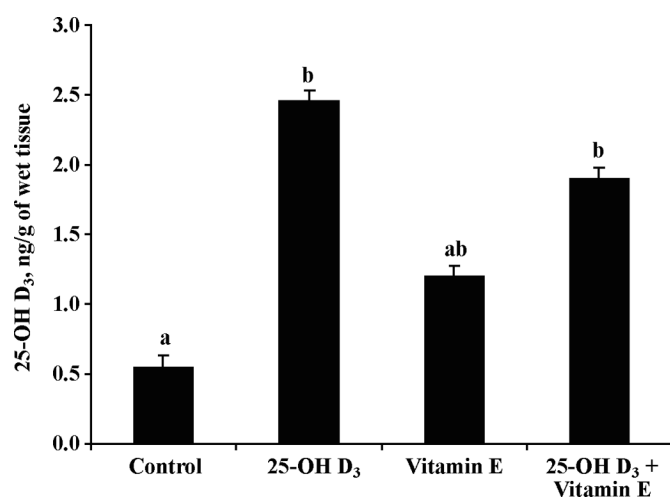


**Figure 2.** Calcium concentration in LM for heifers in control group [no 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>) or vitamin E], 25-OH D<sub>3</sub> group (500 mg of 25-OH D<sub>3</sub> as an oral bolus 7 d before slaughter), vitamin E group (1,000 IU of vitamin E for 104 d before slaughter), or 25-OH D<sub>3</sub> + vitamin E group (25-OH D<sub>3</sub> and vitamin E). Error bars represent SEM. <sup>a,b</sup>Means not bearing a common letter differ ( $P \geq 0.10$ ).





**Figure 3.** Concentration of 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>) in plasma for heifers in control group (no 25-OH D<sub>3</sub> or vitamin E), 25-OH D<sub>3</sub> group (500 mg of 25-OH D<sub>3</sub> as an oral bolus 7 d before slaughter), vitamin E group (1,000 IU of vitamin E for 104 d before slaughter), or 25-OH D<sub>3</sub> + vitamin E group (25-OH D<sub>3</sub> and vitamin E). No differences exist ( $P > 0.05$ ) at the beginning of the study (baseline) or on the day that 25-OH D<sub>3</sub> was administered (7 d before slaughter). Error bars represent SEM. <sup>a,b</sup>Means from 1 d before slaughter not bearing a common letter differ ( $P < 0.001$ ).



**Figure 4.** The 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>) concentration in LM for heifers in control group (no 25-OH D<sub>3</sub> or vitamin E), 25-OH D<sub>3</sub> group (500 mg of 25-OH D<sub>3</sub> as an oral bolus 7 d before slaughter), vitamin E group (1,000 IU of vitamin E for 104 d before slaughter), or 25-OH D<sub>3</sub> + vitamin E group (25-OH D<sub>3</sub> and vitamin E). Error bars represent SEM. <sup>a,b</sup>Means not bearing a common letter differ ( $P < 0.005$ ).

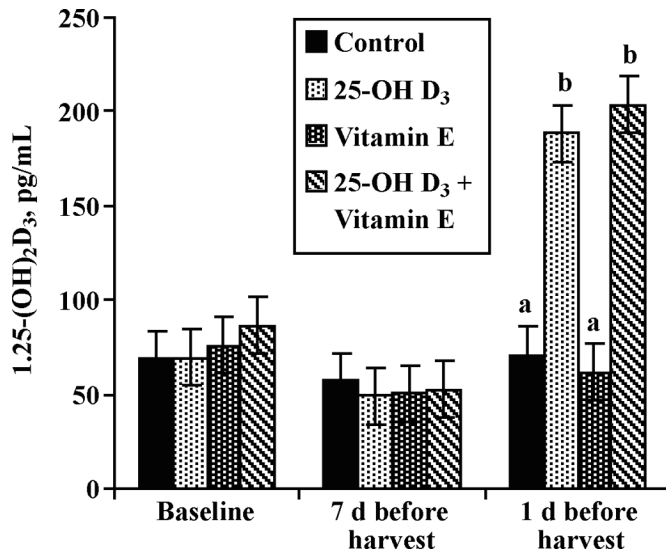
tracted 2 times with n-hexane. The extracts were combined and dried in a Savant SPEEDVAC (Thermo Fisher Scientific, Ashville, NC). The residue was suspended with methanol:chloroform:water (70:25:5; vol:vol:vol), which was also the mobile phase for HPLC analysis. Homogenates and  $\alpha$ -tocopherol standards (Sigma) to make a calibration curve were applied to a C-18 3  $\mu$  250 mm  $\times$  4.6 mm column (Alltech Associates, Inc., Deerfield, IL). The flow rate of the mobile phase was 1.5 mL/min. The detection wavelength was 280 nm.

### Warner-Bratzler Shear Force

Warner-Bratzler shear force values were analyzed to determine tenderness of beef. Steaks were thawed for 24 h at 4°C. An industrial broiler (model CNO2, General Electric, Chicago Heights, IL) was preheated to medium high heat, and the adjustable grill grate was set 11.2 cm below the heat source. Steaks were broiled to an internal temperature of 35°C, turned, and broiled until the internal temperature reached 71°C as determined by a Pyrex digital probe oven thermometer (Cooks Emporium, Ames, IA). Each tray of steaks was wrapped in plastic wrap, and all steaks were stored overnight at 4°C. The following morning, the steaks were allowed to warm to the ambient temperature (approximately 22°C), and six 1.27-cm cores were cut from each steak parallel to the muscle fibers (AMSA, 1995). A texture analyzer (model TA-XTi, Texture Technologies Corp., Scarsdale, NY) fitted with a Warner-Bratzler cutting blade was used to cut the cores perpendicular to the muscle fibers at a penetration speed of 3.3 mm/s. The maximal force necessary to shear each core was recorded, and the values for the 6 cores for each steak were averaged for statistical analysis.

### SDS PAGE and Western Blotting

Samples were prepared for electrophoresis according to the procedure outlined by Huff-Lonergan et al. (1996b). The extent of postmortem proteolysis in beef was determined by SDS PAGE and Western blotting. Meat samples from each heifer aged 3, 7, and 14 d were prepared for quantification of the 30-kDa degradation product of troponin-T that results from postmortem proteolysis (Huff-Lonergan et al., 1996a). Protein degradation products were separated by PAGE. Gels were 15% 100:1 acrylamide:bis-acrylamide (wt/wt). Every gel was loaded with 20  $\mu$ g per lane of each sample, a standard for developing the band density ratios, and a molecular weight marker to ensure that the protein moved through the gel appropriately. The standard used for this study was a 14-d sample from the control group. After the tracking dye reached the end of the gel, the proteins were transferred to a membrane for Western blotting (Huff-Lonergan et al., 1996b). The 30-kDa degradation product was detected by using antibody JLT-12 (Sigma Aldrich, St. Louis, MO) diluted 1:5,000 as the



**Figure 5.** Concentration of 1,25-dihydroxyvitamin D<sub>3</sub> [1,25-(OH)<sub>2</sub>D<sub>3</sub>] in plasma for heifers in control group (no 25-OH D<sub>3</sub> or vitamin E), 25-OH D<sub>3</sub> group (500 mg of 25-OH D<sub>3</sub> as an oral bolus 7 d before slaughter), vitamin E group (1,000 IU of vitamin E for 104 d before slaughter), or 25-OH D<sub>3</sub> + vitamin E group (25-OH D<sub>3</sub> and vitamin E). No differences ( $P > 0.05$ ) existed at the beginning of the study (baseline) or on the day that 25-OH D<sub>3</sub> was administered (7 d before slaughter). Error bars represent SEM. <sup>a,b</sup>Means from the day before slaughter not bearing a common letter differ ( $P < 0.001$ ).

primary antibody and antibody A-2554 (Sigma Aldrich) diluted 1:3,333 as the secondary antibody. The ECL-Plus chemiluminescent system (Amersham Biosciences, GE Healthcare, Piscataway, NJ) was used to detect troponin-T. Membranes were visualized by using a 16-bit megapixel charge-coupled device camera (FluorChem8800, Alpha Innotech Corp., San Leandro, CA) and FluorChem IS-800 software (Version 3, Alpha Innotech Corp.). The band density ratios of the 30-kD degradation product of troponin-T in each sample relative to the reference standard were used to determine the extent of troponin-T degradation and therefore the extent of postmortem proteolysis relative to samples from other treatments in this experiment.

### 2-Thiobarbituric Acid-Reactive Substances

The 2-thiobarbituric acid-reactive substances (TBARS) indicate the extent of fatty acid oxidation of meat samples and were quantified by a distillation method (Koniecko, 1985). Briefly,  $10 \pm 0.02$  g of ground steak was weighed into round bottom flasks. The following were added to each flask: 97.5 mL of distilled water, 2.5 mL of 12 M hydrochloric acid:distilled water (1:2, vol/vol), Dow Antifoam C Emulsion (Sigma-Aldrich):distilled water (1:1; vol/vol), and 5 to 7 boiling beads. The flasks were placed over burners, and a distillation apparatus was attached to each flask. The con-

tents of the flasks were brought to a moderate boil, and 50 mL of distillate was collected. Five milliliters of distillate was pipetted into duplicate tubes, and 5 mL of 20 mM TBA reagent [0.1442 g of 2-thiobarbituric acid (Sigma-Aldrich) dissolved in 50 mL of distilled water] was added to each tube. A standard curve consisting of 0, 2, 4, 6, 8, and  $10 \times 10^{-8}$  M malondialdehyde (Fluka, St. Louis, MO) was run with the samples for determining TBARS concentration and therefore oxidation in samples. Samples and standards were capped firmly and put in a water bath at 100°C for 35 min. Samples and standards were allowed to cool for 10 min before the absorbance was read at a wavelength of 532 nm. Absorbance was recorded, and milligrams of TBARS per kilogram of beef was calculated as the TBARS score.

### Statistical Analysis

All data were analyzed as a  $2 \times 2$  factorial design with 2 dosages of 25-OH D<sub>3</sub> (0 or 500 mg) and 2 dosages of vitamin E (0 or 1,000 IU), and the MIXED procedure (SAS Inst. Inc., Cary, NC) was used to conduct ANOVA. Animal was the experimental unit for all measures. Warner-Bratzler shear force and troponin-T degradation were analyzed as a repeated measure with aging day as the repeated variable. Least squared means were computed for all fixed effects and separated by using pairwise *t*-tests (PDIF of SAS) when a significant *F*-test ( $P < 0.05$  unless otherwise noted) was detected.

## RESULTS AND DISCUSSION

### Feedlot Performance

No differences among treatments were observed in feedlot performance of heifers in the current study (Table 1). Initial weight, final weight, DMI over the whole feeding period and during the last week of the study, ADG, and G:F did not change with dietary supplementation of 25-OH D<sub>3</sub>, vitamin E, or both. Feed intake is of particular interest, because, in studies using vitamin D<sub>3</sub>, cattle tend to consume less feed as their plasma calcium concentrations are increased (Karges et al., 1999, 2001). Similar to the findings of Wertz et al. (2004), the current study indicates that the 500-mg dosage of 25-OH D<sub>3</sub> does not significantly affect feed intake, ADG, or market weight like vitamin D<sub>3</sub> has been shown to do. As shown in Table 1, final weight, ADG, and DMI during the last week before slaughter were numerically decreased in the groups that received 25-OH D<sub>3</sub>. If there were more replicates, these findings could prove significant. Producers usually are not willing to use a procedure that will make cattle less efficient or decrease market weight, which both affect profitability, so 25-OH D<sub>3</sub> should be used after carefully considering the effects it might have.

### Calcium Concentration in Plasma and in Muscle

Calcium concentration in plasma of heifers was not different at baseline or on the day that 25-OH D<sub>3</sub> was

administered (Figure 1). Six days after 500 mg of 25-OH D<sub>3</sub> or placebo was administered (1 d before slaughter), calcium concentrations in plasma from heifers treated with 500 mg of 25-OH D<sub>3</sub> and a combination of 25-OH D<sub>3</sub> and vitamin E were increased by 20 to 25% ( $P \leq 0.05$ ). The calcium concentrations in plasma at baseline and 1 d before slaughter in groups that were treated with a 1-time bolus of 500 mg of 25-OH D<sub>3</sub> 7 d before slaughter were similar to the plasma calcium concentrations observed when  $5 \times 10^6$  or  $7.5 \times 10^6$  IU of vitamin D<sub>3</sub> was administered for 9 consecutive days (Montgomery et al., 2000) and when  $6 \times 10^6$  IU of vitamin D<sub>3</sub> was administered for 4 or 6 d (Karges et al., 2001). Other researchers have not successfully increased plasma calcium concentrations with 125 mg of supplemental 25-OH D<sub>3</sub> (Foote et al., 2004; Wertz et al., 2004), but the failure to induce hypercalcemia probably resulted because of the lower dosage of 25-OH D<sub>3</sub> that was administered.

Total calcium concentration in LM tended to increase ( $P = 0.10$ ) when 25-OH D<sub>3</sub> was administered alone, but the effect when 25-OH D<sub>3</sub> was administered with vitamin E was negligible (Figure 2). As with plasma calcium concentration, experiments by Foote et al. (2004) did not elicit an increase in muscle calcium. Again, this effect probably resulted because of the lower dosage (125 mg) of 25-OH D<sub>3</sub> that was administered. Wertz et al. (2004) did not find an effect of treatment with 125 mg of 25-OH D<sub>3</sub> on water-soluble calcium concentration in LM, but they did not analyze the muscle for total calcium concentration. The possibility exists that the increase in muscle calcium that resulted in the present study is attributable to the water-insoluble calcium concentration in the muscle. However, the likelihood of that possibility is small because Foote et al. (2004) used the same dosage of 25-OH D<sub>3</sub> as did Wertz et al. (2004) and did not find an effect of treatment on total muscle calcium concentrations. The calcium concentrations in the current study were similar to those obtained by Montgomery et al. (2002) and agreed with published values for expected calcium concentrations in beef according to the USDA (2004).

### 25-OH D<sub>3</sub> and 1,25-(OH)<sub>2</sub> D<sub>3</sub> in Plasma and in Muscle

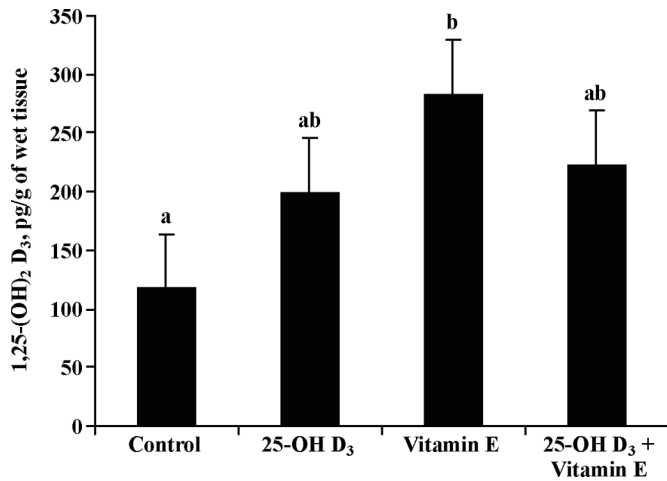
The 25-OH D<sub>3</sub> was increased in plasma and in LM from heifers that were treated with 25-OH D<sub>3</sub> with or without vitamin E ( $P \leq 0.05$ ; Figures 3 and 4, respectively). In plasma, 25-OH D<sub>3</sub> concentrations were 13-fold higher than concentrations of 25-OH D<sub>3</sub> in plasma of heifers not treated with 25-OH D<sub>3</sub>, and these values were approximately 2 times the concentration observed by Wertz et al. (2004) when 62.5 or 125 mg of 25-OH D<sub>3</sub> were administered 21, 7, 4, or 0 d before slaughter. The concentration of 25-OH D<sub>3</sub> in LM was increased when 500 mg of 25-OH D<sub>3</sub> was administered 7 d before slaughter compared with concentrations in heifers in the control group. The concentration of 25-OH D<sub>3</sub> in

the vitamin E group was intermediate to those of the control group and the groups that received 25-OH D<sub>3</sub>. The concentrations of 25-OH D<sub>3</sub> observed in the present study were similar to the concentration observed by Wertz et al. (2004), even though the dosage for the present study was 4 times greater than the largest dosage used by Wertz et al. (2004). Although statistically significant ( $P \leq 0.05$ ), the concentration of 25-OH D<sub>3</sub> in steaks from heifers supplemented with 500 mg of 25-OH D<sub>3</sub> was only about 3 to 4 times the concentration in the control samples. Thus, meat obtained from heifers treated with 25-OH D<sub>3</sub> would be safe to consume. The current upper limit of vitamin D intake is 50 µg of vitamin D<sub>3</sub>/d (Standing Committee on Scientific Evaluation of Dietary Reference Intakes, 1997). The 25-OH D<sub>3</sub> has about 1.4 times the activity of vitamin D<sub>3</sub>; hence, given the concentrations of 25-OH D<sub>3</sub> found in this study, a person would have to consume more than 13.6 kg of meat per day to reach the upper limit. This consumption is not realistic or possible, and thus the small increase in 25-OH D<sub>3</sub> content in beef is not a concern. In fact, consuming beef from cattle supplemented with 25-OH D<sub>3</sub> is safe and may be a reasonable approach to help ensure that consumers meet their daily recommended intake of vitamin D<sub>3</sub> activity, which is equivalent to 5 to 10 µg of vitamin D<sub>3</sub> per day (Standing Committee on Scientific Evaluation of Dietary Reference Intakes, 1997).

Concentrations of 1,25-(OH)<sub>2</sub> D<sub>3</sub> in plasma from heifers treated with 25-OH D<sub>3</sub> were 2 to 2.5 times higher than the concentrations observed in plasma from the control and vitamin E-only groups ( $P < 0.001$ ; Figure 5). The concentrations observed in the present study were similar to the concentrations observed by Montgomery et al. (2000) when 5 or  $7.5 \times 10^6$  IU of vitamin D<sub>3</sub> were provided to cattle and to those observed by Wertz et al. (2004) when 125 mg of 25-OH D<sub>3</sub> was provided to cattle, but lower than the concentrations observed by Foote et al. (2004) when 125 mg of 25-OH D<sub>3</sub> was administered one time before slaughter.

In agreement with previous studies (Montgomery et al., 2000; Foote et al., 2004; Wertz et al., 2004), the concentration of 1,25-(OH)<sub>2</sub> D<sub>3</sub> was not increased significantly in LM when 25-OH D<sub>3</sub> was supplemented ( $P \geq 0.20$ ; Figure 6). In the current study, the 1,25-(OH)<sub>2</sub> D<sub>3</sub> concentration was increased in LM when vitamin E alone was supplemented. Although largely unexplained, this increase could be a result of the antioxidant property of vitamin E acting to protect 25-OH D<sub>3</sub> 1- $\alpha$ -hydroxylase activity in the kidney (Burton et al., 1983). The same result is not expected when 25-OH D<sub>3</sub> is supplemented because transcription of the mRNA for 25-OH D<sub>3</sub> 1- $\alpha$ -hydroxylase would be decreased because of an already higher circulating concentration of 1,25-(OH)<sub>2</sub> D<sub>3</sub> or, possibly because of the elevated concentration of 25-OH D<sub>3</sub> present (Combs, 1992; Shils et al., 1998). Another possible explanation of this increase in 1,25-(OH)<sub>2</sub> D<sub>3</sub> concentration in muscle from heifers treated with vitamin E is that activity of extra-renal 25-



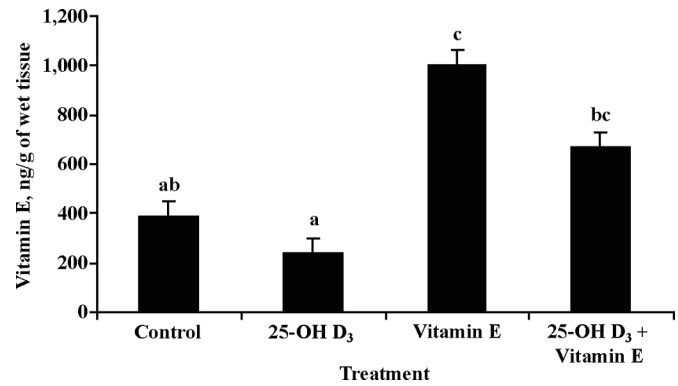


**Figure 6.** The 1,25-dihydroxyvitamin D<sub>3</sub> [1,25-(OH)<sub>2</sub> D<sub>3</sub>] concentration in LM for heifers in the control group (no 25-OH D<sub>3</sub> or vitamin E), 25-OH D<sub>3</sub> group (500 mg of 25-OH D<sub>3</sub> as an oral bolus 7 d before slaughter), vitamin E group (1,000 IU of vitamin E for 104 d before slaughter), or 25-OH D<sub>3</sub> + vitamin E (25-OH D<sub>3</sub> and vitamin E). Error bars represent SEM. <sup>a,b</sup>Means not bearing a common letter differ ( $P < 0.05$ ).

OH D<sub>3</sub> 1- $\alpha$ -hydroxylase might be preserved in muscle by accumulating vitamin E, and, therefore, results in increased concentration of 1,25-(OH)<sub>2</sub> D<sub>3</sub> in muscle. The 25-OH D<sub>3</sub> 1- $\alpha$ -hydroxylase has been shown to be present in smooth muscle (Somjen et al., 2005). Some researchers have considered the possibility of the presence of 25-OH D<sub>3</sub> 1- $\alpha$ -hydroxylase in skeletal muscle, but results currently are not available (M. Hewison, Division of Medical Sciences, The University of Birmingham, Birmingham, UK, personal communication). Unexpectedly, concentrations of 1,25-(OH)<sub>2</sub> D<sub>3</sub> in muscle were intermediate in groups that were supplemented with 25-OH D<sub>3</sub> and were not significantly different from the concentration in muscle from heifers in the control group ( $P \geq 0.20$ ).

### Vitamin E in Muscle

Concentration of vitamin E was determined only in samples aged for 3 d. As expected, the vitamin E concentration was increased in steaks from the LM when vitamin E was supplemented as a top-dress to heifers daily for 104 d before slaughter (Figure 7). The vitamin E concentration in muscle from heifers treated with vitamin E only was higher than the groups not receiving vitamin E ( $P < 0.05$ ). When 25-OH D<sub>3</sub> and vitamin E were administered, the resulting vitamin E concentration was not significantly greater than that of the control group ( $P > 0.10$ ), but was higher than that of the 25-OH D<sub>3</sub> group ( $P < 0.05$ ). This finding agrees with several studies (Harris et al., 2001; Roeber et al., 2001; Rowe et al., 2004) in which  $\alpha$ -tocopherol concentration in meat was increased after supplementation with



**Figure 7.** Vitamin E ( $\alpha$ -tocopherol) concentration in LM for heifers in the control group [no 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>) or vitamin E], 25-OH D<sub>3</sub> group (500 mg of 25-OH D<sub>3</sub> as an oral bolus 7 d before slaughter), vitamin E group (1,000 IU of vitamin E for 104 d before slaughter), or 25-OH D<sub>3</sub> + vitamin E (25-OH D<sub>3</sub> and vitamin E). Error bars represent SEM. <sup>a-c</sup>Means not bearing a common letter differ ( $P < 0.05$ ).

1,000 IU of vitamin E for more than 100 d before slaughter.

### Warner-Bratzler Shear Force

Warner-Bratzler shear force was used to measure the tenderness of steaks from the LM at 3, 7, and 14 d postmortem. Results are shown in Table 2. No significant interaction existed between 25-OH D<sub>3</sub> and vitamin E ( $P > 0.1$ ). Supplementation with 500 mg of 25-OH D<sub>3</sub> or vitamin E tended to improve tenderness of steaks at 14 d postmortem ( $P = 0.08$ ). At 3 d postmortem, no difference in tenderness existed between treatments. By 7 d postmortem, however, steaks from the control and vitamin E treatment groups were more tender than steaks from the 25-OH D<sub>3</sub> or combination groups ( $P \leq$

**Table 2.** Effect of dietary supplementation with 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>), vitamin E, or both on Warner-Bratzler shear force values (kg) of steaks from LM of heifers

Treatment	Postmortem aging, d			SEM <sup>1</sup>
	3	7	14	
Control	3.79 <sup>x</sup>	3.04 <sup>a,y</sup>	3.60 <sup>c,x</sup>	0.31
25-OH D <sub>3</sub>	3.85 <sup>y</sup>	3.61 <sup>b,x</sup>	3.36 <sup>d,x</sup>	0.19
Vitamin E	3.64 <sup>x</sup>	3.05 <sup>a,y</sup>	3.35 <sup>d,x</sup>	0.21
25-OH D <sub>3</sub> + vitamin E	3.76	3.80 <sup>b</sup>	3.95 <sup>c</sup>	0.32
SEM <sup>2</sup>	0.29	0.25	0.25	

<sup>a,b</sup>Treatment means within a postmortem aging period not bearing a common superscript differ ( $P \leq 0.05$ ).

<sup>c,d</sup>Treatment means within a postmortem aging period not bearing a common superscript tend to differ ( $P \leq 0.10$ ).

<sup>x,y</sup>Aging means within a treatment not bearing a common superscript tend to differ ( $P \leq 0.10$ ).

<sup>1</sup>Denotes variation within a treatment across aging periods.

<sup>2</sup>Denotes variation within an aging period between treatments.



**Table 3.** Effect of dietary supplementation with 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>), vitamin E, or both on troponin-T degradation in steaks from LM of heifers<sup>1</sup>

Treatment	Postmortem aging, d			SEM <sup>2</sup>
	3	7	14	
Control	0.92 <sup>a,x</sup>	0.54 <sup>b,y</sup>	1.00 <sup>x</sup>	0.12
25-OH D <sub>3</sub>	1.75 <sup>c,x</sup>	0.80 <sup>a,y</sup>	1.08 <sup>y</sup>	0.19
Vitamin E	1.63 <sup>bc,x</sup>	0.73 <sup>a,y</sup>	0.98 <sup>y</sup>	0.19
25-OH D <sub>3</sub> + vitamin E	1.44 <sup>b,x</sup>	0.54 <sup>b,z</sup>	1.18 <sup>y</sup>	0.21
SEM <sup>3</sup>	0.28	0.15	0.14	

<sup>a-c</sup>Treatment means within a postmortem aging period not bearing a common superscript differ ( $P \leq 0.10$ ).

<sup>x-z</sup>Aging periods within treatments not bearing a common superscript tend to differ ( $0.05 < P \leq 0.10$ ).

<sup>1</sup>Values are ratios of densitometry of the 30-kDa degradation product compared with a control sample aged for 14 d.

<sup>2</sup>Denotes variation within a treatment across aging periods.

<sup>3</sup>Denotes variation within an aging period between treatments.

0.05). Generally, steaks are considered very tender if the shear force is less than 4 kg (Shackelford et al., 2001). In the current study, all of the steak samples, regardless of treatment, already were very tender, even at 3 d postmortem. Wertz et al. (2004) did not observe a decrease in penetration shear force of steak samples after treatment with 125 mg of 25-OH D<sub>3</sub>. Although the same statistical analysis was not performed, the data obtained by Foote et al. (2004) seem to agree with the current finding that 25-OH D<sub>3</sub> enhanced the effectiveness of aging according to characteristics that indicate tenderness. The 25-hydroxyvitamin D<sub>3</sub> did not improve tenderness to the same extent observed when vitamin D<sub>3</sub> is administered (Swanek et al., 1999; Montgomery et al., 2000, 2002; Karges et al., 2001). In the current study, the group treated with vitamin E for 104 d had lower shear force values after 14 d compared with steaks from cattle in the control group, which agrees with a study in which steaks from cattle treated with vitamin E for 125 d were more tender than steaks that were not treated with vitamin E for 125 d (Harris et al., 2001). Interestingly, treating with both supplemental vitamin E and 25-OH D<sub>3</sub> did not improve tenderness of beef.

### SDS PAGE and Western Blotting

At 3 d postmortem, troponin-T degradation in steaks from heifers treated with 500 mg of 25-OH D<sub>3</sub> 7 d before slaughter or 1,000 IU of vitamin E for 104 d before slaughter was nearly 2 times that in steaks from control animals (Table 3). No significant interaction existed between 25-OH D<sub>3</sub> and vitamin E ( $P > 0.1$ ). Degradation in muscle from heifers treated with both 25-OH D<sub>3</sub> and vitamin E was intermediate to degradation in steaks from the control and single treatment groups. The amount of degradation product was decreased at 7 d postmortem in all treatment groups. At 14 d postmortem, the amount of degradation product was higher than at 7 d postmortem but lower than at 3 d postmor-

**Table 4.** Effect of dietary supplementation with 25-hydroxyvitamin D<sub>3</sub> (25-OH D<sub>3</sub>), vitamin E, or both on the concentration of 2-thiobarbituric acid-reactive substances<sup>1</sup> in steaks from LM of heifers

Treatment	Postmortem aging, d			SEM <sup>2</sup>
	3	7	14	
Control	1.02 <sup>a</sup>	1.08 <sup>a</sup>	1.16 <sup>a</sup>	0.2
25-OH D <sub>3</sub>	1.07 <sup>a</sup>	0.95 <sup>a</sup>	1.20 <sup>a</sup>	0.25
Vitamin E	0.67 <sup>b</sup>	0.53 <sup>b</sup>	0.49 <sup>b</sup>	0.08
25-OH D <sub>3</sub> + vitamin E	0.26 <sup>b,x</sup>	0.51 <sup>b,y</sup>	0.61 <sup>b,y</sup>	0.08
SEM <sup>3</sup>	0.24	0.11	0.13	

<sup>a,b</sup>Treatment means within a postmortem aging period not bearing a common superscript differ ( $P \leq 0.05$ ).

<sup>x,y</sup>Aging means within a treatment not bearing a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Concentration = milligrams of malondialdehyde/kilogram of meat.

<sup>2</sup>Denotes variation within a treatment across aging periods.

<sup>3</sup>Denotes variation within an aging period between treatments.

tem. One explanation for this phenomenon could be that the 30-kDa degradation product, which is measured to determine postmortem proteolysis, is also a substrate for other proteases. As the proteases utilize the 30-kDa degradation product, the total amount of product measured decreases. Most other studies show an increase in troponin-T degradation with aging (Montgomery et al., 2000, 2002, 2004; Foote et al., 2004; Wertz et al., 2004), but that increase was not evident in the current trial. Montgomery et al. (2004) showed that, at 14 d postmortem, treatment with  $0.5$  to  $5.0 \times 10^6$  IU of vitamin D<sub>3</sub> increased troponin-T degradation compared with controls. Treatment with 25-OH D<sub>3</sub> or vitamin E seemed to increase the rate of degradation because the greatest increase in product from postmortem proteolysis was present at just 3 d postmortem. Many of the studies that have investigated postmortem proteolysis did not analyze samples at 3 d postmortem. Rather, most have 7-, 14-, and 21-d aging periods. Therefore, the early dynamics observed in this study have not been fully investigated. Data obtained in the current study agree with proteolysis data from samples aged 7 and 14 d postmortem in other studies (Montgomery et al., 2000, 2004).

### 2-Thiobarbituric Acid-Reactive Substances

The TBARS were quantified after steaks were aged for 3, 7, and 14 d. As expected, steaks from heifers treated with vitamin E, with or without 25-OH D<sub>3</sub>, had lower concentrations of TBARS than did steaks from heifers not treated with vitamin E (Table 4). Concentrations of TBARS in steaks within each treatment were similar for all aging periods except in the combination group, which exhibited less oxidation at 3 d than after 7 or 14 d of aging ( $P = 0.05$ ). The concentration of TBARS likely did not increase in most treatments with aging because steaks were vacuum packaged before aging, so limited oxygen was present. The results of TBARS and Warner-Bratzler shear force analyses in the present

study are in agreement with the findings of Harris et al. (2001) who found that a decrease in TBARS resulted in less oxidation of calpains and, therefore, increased postmortem proteolysis and, possibly, improved tenderness.

Results from the current experiment indicate that 500 mg of 25-OH D<sub>3</sub> administered as a single bolus 7 d before slaughter has the potential to alter characteristics that may improve tenderness of steaks from the LM of beef heifers. Five hundred milligrams of 25-OH D<sub>3</sub> was as effective in the current study as 0.5 to 7.5 × 10<sup>6</sup> IU of vitamin D<sub>3</sub> was in previous research at increasing plasma calcium and improving the tenderness of beef without leaving a high concentration of vitamin D<sub>3</sub> or its metabolites in muscle. In fact, the concentrations of 25-OH D<sub>3</sub> found in steaks from the LM may be beneficial as a dietary source of vitamin D for consumers. Additionally, results from the current study indicate that vitamin E also enhances the effect of aging on tenderness of beef. Surprisingly, the effects of vitamin E and 25-OH D<sub>3</sub> were not additive.

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